REMARKS

Claims 1-9, 13, 15, 18-25, 58, 97, 125 and 262-307 are pending and under examination in the subject application. No amendments to the claims are being made with this reply.

Rejections under 35 U.S.C. §103(a)

Claims 1-4, 6-9, 13, 15, 19-22, 24-25, 58, 97, 125, 262-272, 276-284 and 287-307 are rejected as being unpatentable over Wabl et al., U.S. Patent 5,885,827 ("Wabl") in view of Muramatsu et al., *Cell, 102*: 553-563 (2000) ("Muramatsu") as evidenced by Martin et al., *PNAS*, 99:12304-8 (2002) ("Martin").

Claims 5 and 23 are rejected as being unpatentable over Wabl in view of Muramatsu, as evidenced by Martin, and further in view of Wang et al., U.S. Patent Application Publication No. 2003/0119190 ("Wang").

Claims 273, 274 and 275 are rejected as being unpatentable over Wabl and Muramatsu, as evidenced by Martin, and further in view of Griffiths et al., U.S. Patent 5,885,793 ("Griffiths").

Claims 18, 285 and 286 are rejected as being unpatentable over Wabl and Muramatsu, as evidenced by Martin, and further in view of Honjo et al., U.S. Patent 6,815,194 ("Honjo").

Applicants respectfully traverse these rejections for reasons set forth below.

Applicants refer the Examiner to the attached Declaration under 37 C.F.R. §1.132 of Michael S. Neuberger, Ph.D. (5 pages) and his attached Curriculum Vitae (2 pages) and to the attached Declaration under 37 C.F.R. §1.132 of Matthew D. Scharff, M.D. (5 pages) and his attached Biographical Sketch (4 pages).

In his Declaration, Dr. Neuberger indicates that "In 2001 (as well as well before), somatic hypermutation (SHM) was known to involve a programmed process of mutation of variable regions of rearranged immunoglobulin genes that creates additional diversity within an expanding clone of B cells responding to an antigen. Specifically, following antigen recognition by B cells, a B cell enters the germinal center of peripheral lymphoid organs to become a centroblast B cell. In the germinal center, SHM occurs at rates of 10^{-5} to 10^{-3} mutations per base pair per generation, which is ~ 1 million-fold higher than the spontaneous rate of mutation in

most other genes. Generally, the mutations are single base substitutions, with occasional insertions and deletions. While mutations occur throughout the rearranged V regions, the mutations are preferentially targeted to "hot spots" having the sequence WRCY (W=A or T, R=A or G, and Y=T or C) or WA motifs. Transition mutations arise more frequently than transversion mutations (see, e.g., Diaz et al., *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, 356(1405): 67-72 (2001))."

Dr. Neuberger then indicates that "In 2001 (as well as well before), class switch recombination ("CSR") (also known as isotype switching) was known to be a mechanism by which the isotype (or class) of an antibody is changed (e.g., from IgM to IgG). During CSR, a portion of the antibody heavy chain locus is removed from the chromosome, and the gene segments surrounding the deleted portion are rejoined to retain a functional antibody gene that produces an antibody of a different isotype. Double-stranded breaks are generated in DNA in or around conserved nucleotide motifs, called switch (S) regions, which are upstream from gene segments that encode the constant regions of antibody heavy chains; these occur adjacent to all heavy chain constant region genes with the exception of the δ-chain (see, e.g., Janeway et al. (eds.), *Immunobiology*, 5th ed., Garland Publishing, New York, NY (2001))."

Dr. Neuberger then states that "Although by 2001 it was well known that SHM and class switch recombination can occur in B cells at a similar stage of differentiation, it was also known at this time that SHM and CSR appeared to be very different and distinct biochemical processes and frequently occurred independently of each other. The evidence that SHM and CSR are independent processes was further supported by experiments showing that different molecules were involved in SHM and CSR. For example, as early as 2001, CSR was found to be perturbed by a deficiency in the enzyme DNA-PK_{CS}, while SHM was unaffected (see, e.g., Bemark et al., *J. Exp. Med.*, *192*(10): 1509-1514 (2000)). However, several molecules were suggested to play some (albeit undefined) role in allowing both SHM and CSR, including activation-induced cytidine deaminase (AID) (see, e.g., Murumatsu et al., *J. Biol. Chem.*, *274*(26): 18470-18476 (1999), and Murumatsu et al., *Cell*, *102*: 553-563 (2000)), mismatch repair (MMR) proteins (see, e.g., Poltoratsky et al, *J. Exp. Med.*, *192*: F27-F30 (2000)), and error-prone DNA polymerases (see, e.g., Poltoratsky et al., *supra*)."

Dr. Neuberger then indicates that "In 2001 (as well as before), it was hypothesized that SHM was the result of a perturbation in the DNA polymerases involved in DNA damage repair, rather than an active DNA damage *per se* (see, e.g., Zeng et al., *Nat. Immunol.*, *2*: 537-541 (2001), Rogozin et al., *Nat. Immunol.*, *2*: 530-536 (2001), and Zan et al., *Immunity*, *14*: 643-653 (2001))."

Dr. Neuberger then states that "By 2001, the main information known regarding the possible molecular function of AID came from its homology to the RNA-editing enzyme APOBEC1 as well as from its purported deaminase activity on free cytidine (Muramatsu et al., *J. Biol. Chem.*, *274*(26): 18470-18476 (1999)),."

Dr. Neuberger then indicates that "Murumatsu et al., *Cell*, *102*: 553-563 (2000) ("the Murumatsu reference"), which is cited in the Office Action, discloses that overexpression of AID in a lymphoma cell line augments antibody class switching from IgM to IgA without cytokine stimulation. The Muramatsu reference also discloses the generation of AID-deficient mice. AID deficiency completely blocked CSR in B cells activated by lipopolysaccharide (LPS) *in vitro* and by antigens *in vivo*. In addition, the Muramatsu reference demonstrates that B cells isolated from AID-deficient mice have failed to undergo the process of immunoglobulin gene somatic hypermutation. Based on these results, the Muramatsu reference hypothesizes that AID is an RNA editing enzyme that requires a co-factor for its activity (Muramatsu reference at page 560, first column, and page 561, first column)."

Dr. Neuberger states that "The disclosure of the Muramatsu reference demonstrates that the AID gene is necessary for somatic hypermutation and class switch recombination, but the Murumatsu reference does not disclose the molecular function of AID or demonstrate that AID is actually involved in the mechanics of SHM and CSR. As discussed above, deficiencies in several other genes had previously been shown to affect somatic hypermutation and/or class switch recombination. In addition, the conclusions drawn by the Murumatsu reference with respect to the potential role of AID in SHM are based solely on the phenotype of AID knock-out mice. While the Murumatsu reference describes experiments in which AID is overexpressed in mouse B cells, it reports only on the effects of AID overexpression on CSR and not SHM."

Dr. Neuberger further states that "Clearly, the disclosure that a specific gene is necessary for a particular process is not at all the converse of showing that overexpression of the corresponding gene product is sufficient to induce the same process. In fact, that is very rarely the case. Thus, for example, many genes are necessary for the development of B lymphocytes as evidenced by B cell deficiency in a variety of mouse strains lacking specific transcription factors or cell surface molecules. However, in extremely few cases (if any) is ectopic expression of one of these gene products sufficient to induce B cell development in normal non-B-lineage cells. So the demonstration that a functional AID gene was necessary in order to allow CSR and SHM in no sense indicated or even suggested that ectopic expression of AID would be sufficient to induce SHM. That was a striking and unanticipated discovery."

Dr. Neuberger further states that "Thus, as of 2001, AID was one of many factors suspected to be involved in SHM and class switch recombination, but was not reported as the key enzyme responsible for induction of SHM. Moreover, at this time, the mechanism of action of activation-induced cytidine deaminase (AID) was entirely unknown, inasmuch as there was evidence suggesting that AID was an RNA editing enzyme (see, e.g., the Murumatsu reference and also Jacobs and Bross *Curr. Opin. Immunol.*, *13*(2): 208-218 (2001)). Indeed, Poltoratsky et al., *supra*, states that the mechanism of action of AID was unclear in 2000, and that V region mutation still occurs in mice and humans with defects in AID."

Dr. Neuberger further indicates that "In 2001, it was not known nor suggested that AID alone is sufficient to induce SHM. In fact, such an idea would have been greeted with significant skepticism because of the complexity of SHM and class switch recombination processes, the essential restriction of these processes to the Ig locus, and the many other factors thought necessary to enable somatic hypermutation and class switch recombination *in vivo*, as described above. Indeed, one of ordinary skill in the art would not have considered that AID alone would be sufficient to induce SHM."

Dr. Neuberger states that "The Applicants' discovery that AID expression alone is sufficient to initiate somatic hypermutation, which is the foundation of the claimed invention, represented a significant shift in the thinking in the art. Specifically, this discovery prompted

many of those of ordinary skill in the art to consider AID a key protein involved in mechanics of SHM."

Dr. Neuberger concludes that "In view of the foregoing, as of 2001 there was no reason to believe that AID directly induces mutations in DNA that lead to SHM and class switch recombination. Moreover, there was no reason to believe that AID is capable of selectively targeting mutation of DNA sequences within the Ig locus. In addition, one usually could not accurately predict the molecular function of a particular protein based solely on the phenotype produced when the gene encoding the protein is disrupted or deleted."

Dr. Scharff makes many similar points in his Declaration, enclosed herewith. In addition, Dr. Scharff further points out that "As of 2001, there were numerous examples of situations in which the phenotype of a gene knock-out (e.g., in a mouse) was difficult to interpret, and even contradicts the phenotype observed when the same gene is overexpressed in a cell or organism. For example, a deficiency in the 5-hydroxytryptamine (5-HT) 1B gene leads to hyperaggressive behavior in mice (see, e.g., Gingrich et al., *Current Opinion in Neurobiology*, *10*: 146-152 (2000)); however, 5-HT antagonists have no affect on aggressive behavior. The Gingrich reference also states that the phenotypes of knockout mice are difficult to interpret due to a variety of factors, such as development and compensatory changes, the influence of the gene knockout on nearby genes, the effect of the genetic background strain, maternal behavioral influences, and pleiotropy. In another example, mice with a complete deficiency in insulin-like growth factor-1 (IGF-1) exhibit postnatal lethality, growth retardation, infertility, and defects in the development of major organ systems (see, e.g., Liu et al., *P.S.E.B.M.*, *223*: 344-351 (2000)). In contrast, conditional deletion of IGF-1 in mouse liver tissue using the Cre-Lox system produces no defects in growth or development (Liu et al., *supra*)."

Applicants note that for subject matter defined by a claim to be considered obvious, the Patent Office must demonstrate that the differences between the claimed subject matter and the prior art "are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains." 35 U.S.C. § 103(a); see also *Graham v. John Deere Co.*, 383 U.S. 1, 148 U.S.P.Q. 459 (1966). The ultimate determination of whether an invention is or is not obvious is based on

certain factual inquiries including: (1) the scope and content of the prior art, (2) the level of ordinary skill in the prior art, (3) the differences between the claimed invention and the prior art, and (4) objective evidence of nonobviousness. *Graham*, 383 U.S. at 17-18, 148 U.S.P.Q. at 467.

Consideration of the aforementioned Graham factors here indicates that the present invention, as defined by the pending claims, is unobvious in view of the cited references.

Regarding the scope and content of the prior art, the Wabl patent discloses a method of performing random mutagenesis of a heterologous target gene using the immunoglobulin hypermutation system. In particular, the method involves transfecting an immunoglobulin-mutator-positive cell with a hypermutation-competent expression vector into which has been cloned a target gene, and allowing the target gene to hypermutate. The Wabl patent defines a "mutator positive cell line" as a cell line containing cellular factors that work in combination with enhancers to induce hypermutation.

The teachings of the Muramatsu reference are discussed in the attached Declarations.

The Wang application discloses a random mutagenesis system wherein a non-oncogenic, replicating vector acts as a vehicle to randomly introduce a construct comprising a hypermutation-inducing element into the genome of a host cell. Introduction of the hypermutation element in the host cell genome induces mutations (e.g., point mutations, small deletions, and/or small insertions) in genes adjacent to the integrated hypermutation element.

The Griffiths patent discloses a method of isolating antibodies directed against self antigens using phage display technology.

The Honjo patent discloses the amino acid sequences of mouse and human AID proteins.

Neither the Wabl patent nor the Muramatsu reference disclose or suggest that AID causes mutations by deaminating DNA, as required by the claims. Indeed, the precise mechanism of action of AID was unknown in the art at the time the priority application was filed, as evidenced by the attached Rule 132 declarations of Michael S. Neuberger, Ph.D., and Matthew D. Scharff, M.D. In this respect, and contrary to the allegations of the Office Action, at the relevant time AID was one of *many* factors suspected to be involved in somatic hypermutation and CSR, and it was not known or suggested that AID alone is sufficient to directly induce the DNA mutations

required for somatic hypermutation (see also, e.g., the attached recent review by Delker et al., *Nature Immunology*, *10*(11): 1147-1153 (2009)).

The attached Rule 132 Declarations demonstrate that, at the time the application was filed, there were numerous examples of situations in which the phenotype of a gene knock-out (e.g., in a mouse) was difficult to interpret, and even contradicts the phenotype observed when the same gene is overexpressed in a cell or organism.

The Office Action contends that "*the combination* of Wabl and Murumatsu et al. would have inherently deaminated DNA" (Office Action at page 3, last paragraph, emphasis added). Applicants maintain that one of ordinary skill in the art would not have been motivated to choose AID for the purposes of inducing mutations via deamination of DNA. As such, one of ordinary skill in the art would not have been motivated to combine the disclosures of the Wabl patent and the Muramatsu reference and arrive at the subject matter of the pending claims. Moreover, one could not accurately predict the function of a particular protein based solely on the phenotype produced when the gene encoding the protein is disrupted or deleted. For this reason, even if, for the sake of argument, one of ordinary skill in the art were motivated to combine the disclosures of the Wabl patent and the Muramatsu reference, he/she would not arrive at the subject matter of the pending claims with a reasonable expectation of success.

Applicants also note that "[i]nherency and obviousness are distinct concepts. *In re* Spormann, 363 F.2d 444, 448, 150 USPQ 449, 452 (CCPA 1966)." W.L. Gore & Associates, Inc. v. Garlock, Inc., 721 F.2d 1540, 1555, 220 USPQ 303, 314 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984). Furthermore, "the inherency of an advantage and its obviousness are entirely different questions. That which may be inherent is not necessarily known. Obviousness cannot be predicated on what is unknown." *In re* Spormann, 363 F.2d 444, 448, 150 USPQ 449, 452 (CCPA 1966). Finally, "[s]uch a retrospective view of inherency is not a substitute for some teaching or suggestion supporting an obviousness rejection. See In re Newell, 891 F.2d 899, 901, 13 USPQ2d 1248, 1250 (Fed. Cir. 1989)." In re Rijckaert, 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993).

None of the secondary references compensates for the deficiencies of the Wabl patent and the Muramatsu reference. In this respect, the Wang application, the Griffiths patent, and the

Honjo patent do not disclose or suggest expressing a transgenic activation induced cytidine deaminase (AID) gene in a eukaryotic cell and expressing the DNA sequence in the cell, wherein AID initiates mutagenesis by deaminating the DNA sequence. Therefore, the Wang application, the Griffiths patent, and the Honjo patent fail to provide the requisite motivation to combine the Wabl patent and Muramatsu reference in the manner set forth in the Office Action.

Considering all of the Graham factors together, applicants maintain that it is clear that the present invention would not have been obvious to one of ordinary skill in the art at the relevant time in view of the combination of cited references. Accordingly, reconsideration and withdrawal of these rejections are respectfully requested.

Supplemental Information Disclosure Statement

In accordance with the duty of disclosure under 37 C.F.R. §1.56, applicants would like to direct the Examiner's attention to the references that are listed on the attached form PTO/SB/08B (2 pages) and attached hereto. All of these references, with the exception of Delker et al., are referred to in the attached Declaration under 37 C.F.R. §1.132 of Michael S. Neuberger, Ph.D. and/or in the attached Declaration under 37 C.F.R. §1.132 of Matthew D. Scharff, M.D. The Poltoratsky reference mentioned in the Declaration is already of record.

CONCLUSIONS

In view of the preceding remarks and attached Declarations under 37 C.F.R. §1.132, applicants respectfully request that the Examiner reconsider and withdraw the rejections in the May 27, 2009 Final Office Action, and earnestly solicit allowance of the claims under examination. If there is any minor matter preventing the allowance of the subject application, the Examiner is requested to telephone the undersigned attorney.

A check for $960.00 is enclosed for a small entity for the $405.00 fee for filing a Request for Continued Examination and the $555.00 fee for a three month extension of time. No other fee is deemed necessary in connection with this reply. However, if any other fee is required to maintain the pendency of the subject application, authorization is hereby given to withdraw the amount of any such fee from Deposit Account No. 01-1785.

Respectfully submitted,

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